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In-Vitro and In-Vivo Assessment of Anti-Microbial Activity of Extracts of Chhota Chirayta and Lauki

AMRIT PAUL[1], MS. RAGINI BUNDELA[2], DR. SOURABH JAIN*[3], DR. KARUNAKAR SHUKLA[4]

[1]PG Scholar, College of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore MP, [2]Associate Professor, College of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore MP, [3]Professor & Principal, Kewalshree Institute of Pharmacy, Indore MP , [4]Professor & Principal, College of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore MP

Corresponding Author, E Mail ID: drsourabh294@gmail.com

ABSTRACT

Medical microbiology studies illnesses caused by microorganisms, including viruses, bacteria, fungi, algae, and protozoa. Antimicrobial agents inhibit growth, while micro-biostatic agents prevent pathogen growth and aid defense mechanisms. Germicides, categorized by mode of action and administration, may show selective toxicity. They have the ability to destroy viruses, bacteria, algae, or fungi as viricides, bacteriocides, or fungicides. The goal of this study is to determine whether extracts from the plants Lauki (Lagenaria siceraria) and Chhota Chirayta (Swertia chirayita) have antimicrobial qualities. The traditional therapeutic use of these two plants dates back a long way and their possible anti-microbial qualities are highly intriguing when it comes to fighting infectious diseases. The purpose of the study is to evaluate and compare the anti-microbial activity of Lauki and Chhota Chirayta extracts against a variety of harmful bacteria both in vitro and in vivo. The outcomes of the in-vitro and in-vivo studies will offer insightful information about the anti-microbial properties of Lauki and Chhota Chirayta extracts, illuminating potential uses in complementary and alternative medicine. The results could lead to the creation of new treatment alternatives for infectious disorders, especially in areas where traditional herbal medicines are extensively used. This study deserves more investigation since it shows promise

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for using natural plant extracts as possible sources of anti-microbial chemicals in the fight against infectious diseases.

Keywords: Medical Microbiology, Chhota Chirayta Extracts, Traditional Therapeutic, Pathogen Growth.

INTRODUCTION

The World Health Organization (WHO) has been supporting traditional medical practices for the past few decades due to their importance to global health care. This is done to increase the acceptance of herbal drugs globally and demonstrate the clinical efficacy of these age-old remedies. [1] The application of phytochemicals and plant extracts, both of which have proven antibacterial properties, can be extremely important for medical interventions [2]. The antibacterial properties of many plants, which result from chemicals produced during the plant's secondary metabolism, make them useful as medications. These goods are recognized by the ingredients that are active in them[3]. The growing recognition that plant-based therapies are natural products that have minimal environmental hazards, no side effects, are easily accessible at reasonable prices, and are biodegradable is driving up demand for them in both developed and developing nations. More than 6000 medicinal plants are used in basic healthcare by traditional practitioners in India, which is the country that produces the most medicinal herbs [4]. Africa, Asia, and America are home to Lagenaria siceraria (Mohl.) Standley fruit, sometimes known as bottle gourds (Syn. L. vulgaris Ser., Cucurbitalegenaria, Linn., L. leucantha Rusbey, belongs to the family Cucurbitaceae). The bottlegourd's primary and most significant usage was as a water carrier. In medicine, it is used as a cough remedy, a purgative, and an antidote to several toxins [5]. The seeds' infusion has been used to treat headaches and chills, and the leaf juice has been used to treat baldness and jaundice [6][7].

Enicostemma axillare (Lam.) Raynal. syn. E. littorale Blume (Family: Gentianaceae), known locally as Mamajaka in Sanskrit and Chota chirayita in Marathi, has long been utilized as a traditional medicine for a variety of ailments [8]. The fresh leaf juice has been used as a cooling agent, a bitter tonic, to treat typhoid fever, and to regulate arthritis, according to an

Ayurvedic literature review. In cases of dropsy, rheumatism, abdominal ulcers, hernias, swellings, itching, and insect poisoning, it is used as a stomachic and laxative as well as a blood cleanser. On boils, the plant paste is administered. It has been claimed that plant extracts have biological properties that include being antidiabetic, anti-inflammatory, stimulant, astringent, diuretic, and beneficial for skin conditions. The herb has diuretic, anthelmintic, astringent, and stimulating qualities [9][10]. It also treats snakebite ethnomedically [11]. The plant is a leucorrhoea remedy. In vitro and in vivo antimalarial activity was demonstrated by the root extract. [12]. The goal of the current investigation was to assess the efficacy of aqueous and organic solvent extracts of Chhota Chirayta and Lauki leaves against a number of bacterial and fungal species that are significant to medicine.

MATERIAL AND METHOD

Preparation of the plant extract

The leaves were dried to a constant weight at 18°C in an enclosed air conditioned research laboratory. The dried leaves were blended to powder to increase the surface area for extraction and divided into two equal parts for both soxhlet and cold extraction procedures. The entire weight of the leaves powder was 100g.

Phytochemical test

Chemical tests performed in the screening and identification of phytocemical constituents in the tested medicinal plants were carried out in extracts as well as powder specimens using the standard procedures.

Maeyer's test

0.355 g of mercuric chloride was dissolved in 60 ml of distilled water.5.0g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Dragendroff's reagent

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water.

Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilledwater.

Both solutions (A&B) were mixed in 1:1 ratio.

Test for alkaloids

About 0.5 to 0.6 g of the aqueous alcoholic plant extract was mixed in 8 ml of 1% HCL,warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendroff's).

Test for steroids

About 0.5 g of aqueous alcoholic extract fraction of each plant was mixed with2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

Test for terpenoids

An aliquot 0.5 ml of aqueous alcoholic extract was mixed with 2 ml of CHCI3in a test tube. 3 ml of concentrated H2SO4 was carefully added to form a layer.

Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.

Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

Test for phytosterol

The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube.

Foam test: 5 ml of the test solution taken in a test tube was shaken well for five minutes.

Olive oil test: Added a few drops of olive oil to 2 ml of the test solution and shaken well.

TEST FOR GLYCOSIDES

Keller-Killani Test: Added 0.4 ml of glacial acetic acid and a few drops of 5% ferricchloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tubecarefully.

Hydroxyanthraquinone Test: To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution.

MIC determination procedure for ethanolic & aqueous extract

The MIC of extracts was determined by diluting different concentrations of A, B, and C, mixed with nutrient broth, and incubated at 37o C for 18-24 hours. The MBC was determined by sub culturing the test dilution on fresh solid medium and incubating again. The lowest concentration of extracts with no visible bacterial growth was considered MBC.

Preparation of nutrient agar medium

Readymade dehydrated medium supplied by Hi Media was used for testing the anti microbial activity of plant extracts. The dehydrated medium was dissolved in 100ml of distilled water and heated to boiling to dissolve the medium completely following the instructions given by manufacturer. The medium was distributed on clean glass tubes and plugged with cotton and sterilized by autoclaving at 15lb/sq. inch pressure at 121ºC for 20 minutes.

Spread plate method

20ml of nutrient agar media was transferred into each petri plate. The petri plates were left undisturbed for 1-2hrs. 100 µl of each pure culture was transferred into petri plates using micro pipette. The pure cultures were evenly spread with the help of sterile bent glass rod. They were kept for incubation for 24hrs.

Procedure for Antibacterial Activity Testing

Drug substances that either suppress or influence the growth of microorganisms are generally analyzed by microbial method. The procedure employed for this testing is Cup plate method or Agar well diffusion method.

Cup plate method

The antibacterial activity of the extracts was determined by using the agar well diffusion technique. Mueller- Hinton agar plates (Himedia, Mumbai) were seededwith 0.1 ml of overnight culture, allowed to incubate for 24hrs. Cups were made in Petri plates using sterile cork borer (0.85 cm) and 50µl of each extract was added into each well. Then bacterial plates were incubated at 37˚ C 24 hrs[13]. Each test compound has got six bores for which zone of inhibition diameter and mean values were determined. Antibacterial activity was determined by measurement of zone of inhibition around each well in plate using zone reader

[14]. Measured inhibition zones were recorded as mean diameter in mm [15]. Gentamycin antibiotic was used as control.

In vivo anti-bacterial activity

Mice were divided into 6groups and each group consists of sixanimals.

Groups were divided shown0 below:

1. Control group Salmonella-infected (SI)
2. Positive control Salmonella-infected + Gentamycin.
3. Treatment group- 1: Aqueous ethanolic extract of *Lagenaria siceraria* 200mg/kg
4. Treatment group-2: Aqueous ethanolic extract of *Lagenariasiceraria* 400mg/kg
5. Treatment group-3: Aqueous ethanolic extract of *Enicostemma littorale* 200mg/kg
6. Treatment group-4: Aqueous ethanolicextract of *Enicostemma littorale* 400mg/kg.

All through the experiment, mice were provided with water that contained streptomycin ($5\ mg\ mL^{-1}$) in order to reduce the level of facultative anaerobic bacteria that normally colonize the mouse intestine. The inhibition of the growth of test organisms in mice was then determined by monitoring *S. typhimurium* in the feces of the mice. Briefly, *S. typhimurium* (JOL 389) was grown overnight in Luria–Bertani broth (Difco), centrifuged, washed in phosphate-buffered saline (PBS) and then diluted into 20% sucrose to achieve a final concentration of 1×10^5 CFU. The SI and SIPG groups exclusively were then inoculated using gavage needle orally with approximately 10^5 CFU of *S. typhimurium* in a 0.1 mL volume. One hour after infection, animals in the SIPG group were orally administered 5 mg (using gavage needle) of the PGPE daily, whereas CON and SI animals were not. Fecal samples were then collected 0, 1, 2, 3, 4 and 5 days after the bacterial suspensions were administered and the numbers of the bacteria per gram of feces were determined.

RESULT AND DISCUSSION

The study examined the effects of a hydroalcholic extract of Lagenaria siceraria utilizing a two-fold serial dilution approach on a range of bacterial strains, ranging from 1000 µg/ml to

32.5µg/ml. The findings demonstrated that Lagenaria siceraria exhibited a minimum inhibitory concentration (MIC) against every bacterial strain in the broth ranging from 250 µg/ml to 500 µg/ml. The outcomes were similar to the positive control, where the MIC ranged from 1.8625 µg/ml to 3.625 µg/ml.Using the cup plate approach, identical results were also seen in the zone of inhibition. We were able to choose S. Typi for our additional antibacterial research because the medication had superior action against a variety of bacteria. The effects of Enicostemma littorale against a variety of bacterial strains were investigated at dose series ranging from 1000 µg/ml to 32.5 µg/ml using the two-fold serial dilution method. The findings demonstrated that Enicostemma littorale exhibited a minimum inhibitory concentration (MIC) against all bacterial strains at 250 µg/ml to 500 µg/ml of the broth. The outcomes were similar to the positive control, where the MIC ranged from 1.8625 µg/ml to 3.625 µg/ml.Using the cup plate approach, comparable results were also seen in the zone of inhibition. We were able to choose S. typi for our additional antibacterial revision because the medication shown improved action against a variety of bacteria.

Phytochemical Analysis

Based on the results of the phytochemical screening, it was determined whether the alkaloids were present by looking for signs of turbidity and/or precipitate formation. In several samples, the color shifted from violet to blue or green, indicating the presence of steroids. A positive outcome was that no reddish-brown colored interface formed when terpenoids were absent. According to Shinado's test, the presence of flavonoids is shown by red coloring. There was a noticeable color shift in the test tube, indicating the presence of tannins. The creation of a brown ring at the junction and the upper layer's transition to a dark green hue verified the phytosterol test result. The two observations below showed the presence of saponins. Stable foam formation validated the test. The test was verified by the creation of a soluble emulsion. The test was validated by the acetic acid layer forming a blue color. The test was validated by the formation of red color. Glycosides were present, according to the first two observations.

Table No1: Phytochemical Analysis

S.No	Phytochemicals	
1	Alkaloids	+

2	Steroids	+
3	Terpenoids	-
4	Flavonoids	+
5	Tannins	+
6	Phytosterol	+
7	Saponin	+
8	Glycosides	+

+, Presence of the compound

-, Absence of compound

Table No 2. Effect of *Lagenaria siceraria* on selected bactrial strains(Two foldserial dilution)

S. No	**Organisms**	***Lagenaria siceraria* Extracts MIC Values µg/ml**	***Enicostemma littorale* Extracts MIC Values µg/ml**	**Standard drug MIC Values µg/ml**
1.	*S.typi*	500	250	3.625
2.	*S. enteritidis*	500	250	3.625
3.	*E.coli*	500	500	3.625
4.	*S. aureus*	500	500	1.8625
5.	*K. pneumoniae*	250	500	1.8625

Standard drug – Gentamycin

Table No: 3: Effect of Lagenaria siceraria on elected fungal strains (Cup Plate method)

S. No	**Organisms**	**Concentrationsextract**	**Zone of inhibition(cm)**				**Standard drug Zone of inhibition values(cm)**	
			Lagenaria siceraria		***Enicostemma littorale***			
			Well 1	**Well 2**	**Well1**	**Well 2**		

Dr. SOURABH JAIN: *Professor & Principal, Kewalshree Institute of Pharmacy, Indore MP.India.*

							Well 1	**Well 2**
1.	*S.typi*	1000 µg/ml	1.2	1.4	1.6	1.1		
		2000 µg/ml	2.1	2.4	2.9	2.1	1.3	1.6
2.	*S. enteritidis*	1000 µg/ml	1.3	1.4	1.7	1.2		
		2000 µg/ml	2.3	2.4	3.2	2.3	1.2	1.7
3.	*E.coli*	1000 µg/ml	1.2	1.1	1.9	1.1		
		2000 µg/ml	2.7	2.1	4.0	2.6	1.8	1.9
4.	*S. aureus*	1000 µg/ml	1.5	1	1.7	1.5		
		2000 µg/ml	2.7	2.3	3.6	2.7	1.8	1.7
5.	*K. pneumoniae*	1000 µg/ml 2000 µg/ml	1.1 2.5	1.5 2.7	1.8 2.9	1.1 2.5	1.5	1.6

***In vivo* anti-bacterial activity**

The effects of an enicostemma littorale extract on SI mice were investigated. On the fourth day of treatment, enicostemma littorale and siceraria at 400 mg/kg completely prevented infection. Only on the fifth day after infection has the full eradication of the infection been noted. The outcomes were contrasted with those of gentamycin, which began to effectively control the infection on the third day of therapy and reached its peak level of eradication on the fourth day.The findings showed that Lagenaria siceraria had an effective inhibitory effect against bacteria starting on the third day in rats at doses of 2 mg/kg and 400 mg/kg. On the

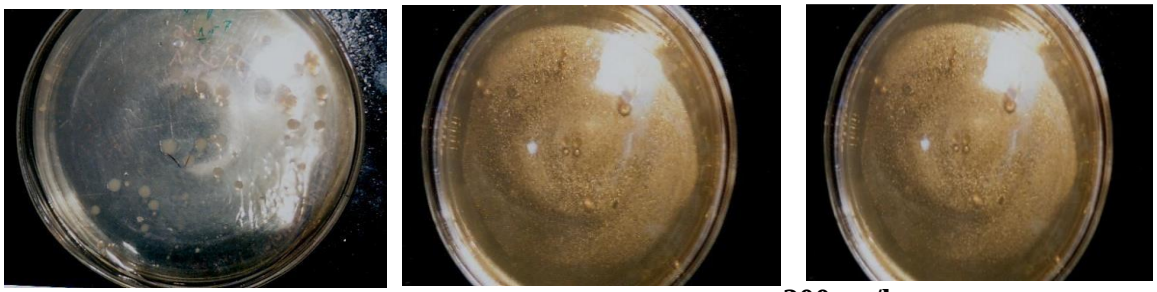
fourth day of treatment, 400 mg/kg of Lagenaria siceraria completely suppressed the infection. On the fifth day following infection, however, the infection was completely eradicated. The outcomes were contrasted with those of gentamycin, which began to effectively control the infection on the third day of therapy and reached its peak level of eradication on the fourth day.

Table No: 4: Effect of Lagenaria siceraria &Enicostemma littoraleagainst s. typiin mice

S. No.	Groups	Organism /Drugs/ Salmonella	Dose mg/kg	Colony Forming Units (c.f.u.) days					
				0	1	2	3	4	5
1.	Control	CMC 0.5%	Required	7.9 ±	9.1 ±	10 ± 0.3	13.6 ±	23.3 ± 0.3	19.5 ± 0.5 x 10^4
	Group		quantity	0.08	0.05 x	x 10^4	.1 x 10^4	x 10^4	
				x 10^4	10^4				
2.	Positive	Gentamycin	2	6 ±	7.8 ±	6.7 ± 0.05	4.1 ±	2.1 ± 0.03 x	Nil
	Control			0.06	0.02 x	x 10^2	0.05 x	10^2	
				x 10^3	10^2		10^2		
3.	Treatment	AAEEL	200	3.6 ±	8.1 ±	6.1 ±	4.8 ±	3.8 ± 0.1 x	2.6 ± 0.3
	group- 1			0.04	0.02 x	0.07 x 10^3	0.03 x	10^3	x 10^3
				x 10^4	10^3		10^3		
4.	Treatment	AAEEL	400	1.6 ±	5 ±	3.4 ± 0.01	1.5 ±	5.2 ± 0.1 x	4.5 ± 0.2
	group- 2			0.09	0.08	x 10^3	0.03 x	10^2	x 10^2
				x 10^4	x 10^3		10^3		
5	Treatme	AAELS	200	2.6 ±	7.1 ±	5.1 ±	4.8 ±	2.8 ± 0.1	2.6 ± 0.3

	nt							x	
	group-3			0.04	0.02 x	0.07 x 10^3	0.03 x	10^3	x 10^3
				x 10^4	10^3		10^3		
6	Treatme nt	AAELS	400	1.6 ±	5.9 ±	4.4 ± 0.01	3.5 ±	2.2 ± 0.1 x	1.8 ± 0.2
	group-4			0.09	0.08	x 10^3	0.03 x	10^2	x 10^2
				x 10^4	x 10^3		10^3		

Figure No: 1: Control, Positive Control, Aqueous ethanolic extract of *Lagenariasiceraria*



200mg/kg

Figure No: 2: Aqueous ethanolic extract of *Lagenariasiceraria* 400mg/kg, *Enicostemma littorale* 200mg/kg and *Enicostemmalittorale* 400mg

CONCLUSION

Plants are gaining popularity as an alternative to synthetic drugs due to their lower incidence of adverse reactions and reduced cost. This study aims to investigate the antibacterial activity of the herb Enicostemma littorale and Lagenaria siceraria against various microbes using the spread plate method. Antibiotics have lost effectiveness due to the development of resistant

bacteria, leading to hypersensitivity, immune-suppression, and allergic reactions. The study suggests that the EtOH extract may have antibacterial activity against Salmonella, but further research is needed to better understand the minor components and determine its antibacterial effect.

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